33 Cont.

17. The method of claim 12 wherein said neural tissue of step (a) is obtained from a mammalian central nervous system.--

REMARKS

Claims 1, 11, and 12 are amended to more particularly define the invention and to more closely correspond to the claims of the co-pending parent application U.S. Ser. No. 08/270,412. New claims 14-17 are also added to more particularly define the invention. Support for the amendments and the new claims exists in the specification as indicated below.

Rejection under 35 U.S.C. §112

Claims 1-13 stand rejected under 35 U.S.C. §112, second paragraph, for lacking proper antecedent basis for the phrase "the tissue". The claims have been amended to correct the lack of antecedent basis. Accordingly, it is believed that the rejection is overcome.

Rejection under 35 U.S.C. §103

Applicants acknowledge the obligation under 37 C.F.R. § 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made. Each claim of the present application was commonly owned at the time made.

Claims 1-10 stand rejected under 35 U.S.C. § 103 as being unpatentable over Reynolds *et al.* (Rest. Neuro. & Neurosci.) in view of Masters *et al.* The authors of the referenced abstract are Brent A. Reynolds and Samuel Weiss. Drs. Weiss and Reynolds are two

of the inventors of the subject application and the inventors of U.S. Ser No. 07/726,812 filed July 8, 1991 (now abandoned in favor of U.S. Ser. No. 08/270,412 filed July 5, 1994) from which the present application claims priority under 35 U.S.C. §120. Accordingly, the reference describes the inventors' own work and was published between the filing dates of the present application and the filing of U.S.S.N. 07/726,812.

The Examiner cites Reynolds *et al.* as teaching "isolating neural stem cells from a donor tissue and proliferating the stem cells by culturing the cells in a medium comprising EGF". However, this teaching is substantially the same as that disclosed in the parent application. Example 5 of 07/726,812, which begins on page 40, line 20 discloses dissociating and proliferating mouse brain cells in a culture medium containing EGF. Accordingly, Reynolds *et al.* is not a proper reference for the purposes used by the Examiner and the rejection of Claims 1-10 under § 103 over this reference should be withdrawn.

Claims 1-10 stand rejected under 35 U.S.C. §103 as being unpatentable over Cattaneo *et al.* taken with Reynolds *et al.* (Soc. for Neurosci. Ab.) or Anchan *et al.* in view of Masters *et al.* Claim 1 has been amended to more clearly set forth that the claimed method is directed to a method of preparing differentiated cells from multipotent neural stem cells capable of producing progeny that are capable of differentiating into neurons, astrocytes, and oligodendrocytes. Support for this amendment is found on page 13, lines 9-18 wherein it is disclosed that the neural cells of the present invention differentiate into these neural cell types. Hence,

the method of the present invention utilizes multipotent neural stem cells.

The hallmark of a stem cell is that it is capable of self maintenance. A cell exhibiting self maintenance is capable of unlimited proliferation and the generation of a large number of progeny. The *in vitro* proliferation of a multipotent neural stem cell typically results in a cluster of cells referred to as a neurosphere. The neurosphere initially consists of two types of undifferentiated cells: multipotent neural stem cells and progenitor cells, which are collectively referred to as precursor cells. "Self maintenance" as defined by Potten & Loeffler [Development 110: 1001-1020 (1990); attached as Exhibit B] is "the ability to maintain its own numbers" and is "a property exclusively of stem cells". The concept of self maintenance is illustrated by the attached diagram labelled Exhibit A. "Multipotent" means that the progeny of the stem cell are capable of differentiating into several neural cell types; for example, neurons, astrocytes and oligodendrocytes. In comparison, a unipotent cell gives rise to only one cell type, for example neurons. A bipotent cell gives rise to two different types of cells; for example, the O-2A progenitor cell is described as a bipotent cell as it is capable of producing progeny that differentiate into either oligodendrocytes or type 2 astrocytes. Applicants' specification sets forth the definition of "stem cell" on page 8, lines 25 to 30. Also, Example 1 on pages 15-16, describes the proliferation of multipotent neural stem cells and the procedure whereby proliferation can be repeated continuously without differentiation. Thus, the applicants have developed culturing methods that allow for the isolation of multipotent neural stem cells from primary tissue

composed of a population of various cells, including differentiated cells and undifferentiated cells that are capable of limited proliferation but are not stem cells. A "progenitor cell" is capable of a limited number of divisions before differentiation. By altering the culture conditions, the progeny of the multipotent neural stem cells can be induced to differentiate into neurons, astrocytes and oligodendrocytes as set forth in step (c) of claim 1.

The method of claims 1 to 10 of the present invention is substantially different from the method described in Cattaneo *et al.* On p. 4, line 16 of the Office Action, the Examiner refers to the cells of Cattaneo *et al.* as "stem cells". However, Cattaneo *et al.* do not demonstrate that the cells exhibit self maintenance (the critical feature of a stem cell). In addition, the cells described in this reference only differentiated into neurons as evidenced by neurofilament staining. Cattaneo *et al.* fail to disclose or suggest how to proliferate cells that differentiate into cell types other than neurons. Thus from this reference, one of ordinary skill in the art is provided no guidance on how to proliferate <u>multipotent neural stem cells</u> (i.e. cells capable of self maintenance and hence unlimited proliferation and subsequent differentiation of the progeny of such cells into neurons, oligodendrocytes, and astrocytes). Cattaneo *et al.* at best demonstrate increased proliferation of <u>unipotent neuronal progenitor cells</u> *in vitro* for a limited period of time (9 days). In addition, Figure 1, p. 763 of Cattaneo *et al.* indicates that the cells were also differentiating during that period, as illustrated by the increase in neurofilament staining cells.

Additionally, the method Cattaneo *et al.* use to induce cell proliferation requires that the cells be pre-exposed to serum (see

legend to Figure 1), as was typically done in methods of culturing neural cells at the time of the present invention. The applicants have developed methods for culturing, proliferating, and differentiating multipotent neural stem cells and progenitor cells in completely defined culture conditions (i.e. without any exposure to serum). Claim 14 has been added to set forth this distinguishing feature of proliferating multipotent neural stem cells in defined culture conditions. Claim 7 and new claim 15 set forth the feature of differentiating the cells in defined culture conditions. Support for new claim 14 is found on page 11, lines 8-13, and in Example 1 where the culture medium used for dissociation and proliferation of the stem cells is described as a defined, serum-free medium. Support for new claim 15 is found on page 18 line 29 to page 19 line 4 where culture conditions allowed differentiation in a defined, serum-free medium. Support is also found on page 17, lines 15 to 29 where differentiation methods are described using complete medium with or without 1% FBS. While the present invention also provides methods for differentiating precursor cells in the presence of serum, culture methods that use completely defined conditions, especially when the cells are to be put to therapeutic uses, are highly desirable. Cattaneo *et al.* fail to teach or suggest how this can be accomplished.

With regards to Reynolds *et al.*, it is submitted that it is not a proper reference for the purpose of the Examiner's rejection for the reasons set forth above.

The Examiner also cites Anchan *et al.* as rendering the present invention obvious when combined with the teachings of Cattaneo *et al.* Anchan *et al.* teach that neuroepithelial cells of the

rat retina retain mitotic activity for "several days" when cultured as aggregates of more than 5 cells per cluster. However, after this limited amount of mitotic activity, the cells begin to differentiate as indicated by immunohistochemistry for various neuron specific antigens. The Examiner states that "Anchan *et al.* also teaches that EGF or NGF increases neural cell proliferation...". However, one skilled in the art can only glean from the limited disclosure of Anchan *et al.*, that a method of culturing unipotent neuronal progenitor cells is described (i.e. cells capable of differentiation only into neurons). Anchan *et al.* do not teach a method of preparing differentiated cells by proliferating multipotent neural stem cells that are capable of self maintenance and differentiation into neurons, astrocytes, and oligodendrocytes. Anchan *et al.*, at best discloses that "increased cell proliferation" occurs in the presence of EGF and NGF. However, as noted above, Anchan *et al.* fail to describe methods of culturing multipotent neural stem cells. Moreover, there is no teaching whatsoever on the culture conditions used. Thus Anchan *et al.* is not an enabling disclosure, and cannot, even if combined with Cattaneo *et al.* teach or suggest the presently claimed methods., i.e. proliferation of multipotent neural stem cells and subsequent differentiation of stem cell progeny that are capable of differentiating into neurons, astrocytes, and oligodendrocytes. Additionally, the combination of Anchan *et al.* with Cattaneo *et al.* fails to teach or suggest proliferation of multipotent stem cells in a defined culture medium wherein the multipotent stem cell is not exposed to serum *in vitro* as set forth in claim 14.

Thus, at the time the culture method of claim 1 was invented, one skilled in the art seeking to develop a culture method

for a multipotent neural stem cell that can be proliferated to generate large numbers of undifferentiated cells that can be differentiated at will upon a change in culture conditions, into neurons, astrocytes, and oligodendrocytes, would not turn to Cattaneo *et al.* and Anchan *et al.* for guidance in doing so. This is because Cattaneo *et al.* and Anchan *et al.* only disclose limited proliferation, and moreover, they only disclose the differentiation of cells into neurons. Thus Anchan *et al.* and Cattaneo *et al.*, even if combinable, fail to disclose or suggest all the elements of the claimed invention, in that they fail to disclose or suggest proliferation and differentiation of multipotent neural stem cells.

The Examiner also cites Masters *et al.* as rendering the present invention obvious when combined with Cattaneo *et al.* because the reference teaches that growth factors accelerate the differentiation of neural cells. However, this reference is only concerned with the differentiation of glial progenitor cells into oligodendrocytes. Thus, the reference does nothing to cure the deficiencies of the other cited references in that it fails to disclose methods for proliferating and differentiating multipotent neural stem cells which give rise to progenitors which give rise to neurons, astrocytes and oligodendrocytes.

Claim 11 stands rejected under 35 U.S.C. § 103 as being unpatentable over Cattaneo *et al.* taken with Reynolds *et al.* The comments made above with respect to the Reynolds *et al.* reference not being a prior art reference for the purposes used by the Examiner are reincorporated here. Accordingly, it is believed that the rejection of claim 11 should be withdrawn.

Claims 12-13 stand rejected under 35 U.S.C. § 103 as being unpatentable over Gensburger *et al.* taken with Yamada *et al.*

Unlike the culture methods set forth in claims 12 to 13, there is no indication that the methods of Gensburger *et al.* result in the proliferation of multipotent neural stem cells that are capable of generating precursor cells including progenitors that can differentiate into neurons, astrocytes and oligodendrocytes. Instead, the reference states that the cultures produced "appear virtually devoid of glial cells" (see last sentence on p. 3 of Gensburger). Gensburger *et al.* teach cell culture methods that result in minimal proliferation of unipotent neuronal precursor cells prior to differentiation into neurons.

The growth factor used in the Gensburger culture medium was bFGF. They could show no effects when other growth factors such as EGF were used (see Table 1 of Gensburger). This suggests that Gensburger *et al.* were not dealing with the same population of cells that are cultured using the methods claimed in the subject application. The culture conditions described by Gensburger *et al.* are also substantially different. They use only one culture medium to proliferate neuronal progenitor cells and to effect the differentiation of these cells into neurons. In addition, their cells are exposed to serum and thus are not cultured in defined conditions. The method of claim 12 recites the culturing of cells in a first culture medium that induces proliferation then culturing the cells in a second culture medium that induces differentiation. With the method of claim 14, the first culture medium is defined (i.e. free of serum).

The Examiner states that "Gensburger *et al.* does continue to teach the differentiation of the precursor cells by contacting the cells with a substrate". However, there is no description in the reference where **differentiation is induced** by contacting the cells with a specific substrate. Instead, the cells of Gensburger *et al.* are proliferated (albeit minimally) and differentiated in the same culture medium and poly(L-lysine)-precoated petri dish. The reference fails to teach changing culturing conditions to induce the cells to change from a proliferative state to a differentiative state.

The Examiner also states that "Yamada *et al* teaches that as a component of the extracellular matrix, fibronectin, is well known in the art to modulate the differentiation of a variety of cell types" including neural cells. The Examiner then concludes that "one would have a reasonable expectation of using fibronectin as the substrate to differentiate neural precursor cells." However, according to the Sieber-Blum *et al.* reference (1981) cited by Yamada *et al.*, undifferentiated neural crest cells and glial and neuronal cells **lack** fibronectin (see Table 1 of Yamada). Therefore, it would not be obvious to add it to cultures used for embryonic CNS neural tissue, as the substance appears to be lacking in their immediate embryonic environment. Evidence from Sieber-Blum *et al.* indicates that fibronectin may influence differentiation of neural crest cells in the trunk region — producing adrenergic cells. This would be in keeping with a role for fibronectin in influencing **peripheral** neurons as they migrate throughout the body. The neural crest region is the site of origin of peripheral neurons, not CNS neurons. Claim 17 has been added to further define that the cells cultured are multipotent neural stem cells derived from central

nervous system tissue. Support for this amendment is found throughout the specification, and particularly in Example 1 where the cells cultured are obtained from brain tissue. There is no evidence in the Yamada paper that fibronectin would influence differentiation of CNS neurons. Therefore, there is no reason for one of ordinary skill in the art to believe that fibronectin would have an effect on CNS neural stem cells.

In view of the amendments to the claims, and for the above reasons, it is believed that the rejections under § 103 are overcome.

For the above reasons, it is believed that this application is in condition for allowance. Such action is respectfully requested. The Examiner is also requested to telephone the undersigned if, in the Examiner's opinion, a telephone conference will facilitate the prosecution of this application.

Respectfully submitted,

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